Supplemental Information

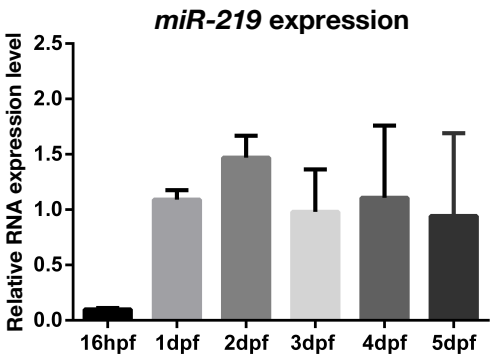

Figure S1

Figure S1 (related to Figure 2).

***miR-219* Expression Across Developmental Stages**

Graph showing relative *miR-219* expression levels during embryonic and larval development measured by quantitative PCR (*n*=3 biological replicates consisting of 15-20 larvae for each measurement).

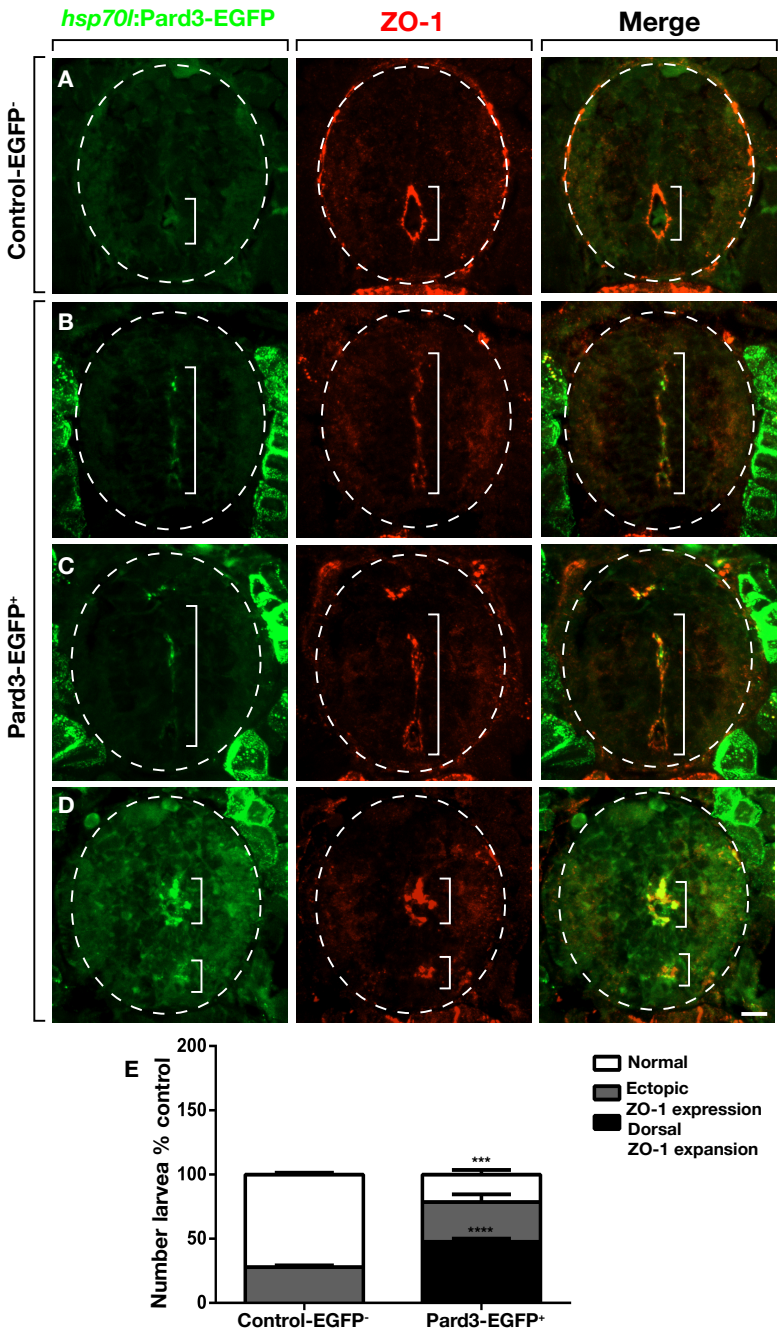

Figure S2

Figure S2 (related to Figure 7).

Pard3-EGFP Overexpression Phenocopies *miR-219* Loss of Function

Representative spinal cord transverse images of 3 dpf heat-shocked control and heat-shocked *Tg(hsp70l:pard3-EGFP)* larvae processed for ZO-1 immunohistochemistry with dorsal to the top. (A) Control non-transgenic larva. ZO-1 is restricted to the central canal. (B and C) Examples of heat-shocked transgenic larvae showing strong Pard3-EGFP expression outside the spinal cord (outlined) and co-localized with ZO-1 to an apparent primitive lumen extending into dorsal spinal cord. (D) Example of a heat-shocked larva with an apparent double lumen (ectopic class) with co-localized Pard3-EGFP and ZO-1. (E) Graph showing number of larvae having dorsal expansion or ectopic co-localization of Pard3-EGFP (n=12 each for control and experimental) ***P=0.0002, ****P<0.0001. Scale bar equals 10 μm.

Supplemental Experimental Procedures

Zebrafish Husbandry

Embryos were produced by pair-wise mating and kept at 28.5°C in egg water or embryo medium (15 mM NaCl, 0.5 mM KCl, 1 mM $CaCl_2$, 1 mM $MgSO_4$, 0.15 mM KH_2PO_4, 0.05 mM NH_2PO_4, 0.7 mM $NaHCO_3$). Embryos were staged to hours postfertilization (hpf) or days postfertilization (dpf) according to established zebrafish guidelines (Kimmel et al., 1995). The experiments conducted in this study used the following strains of zebrafish: AB, *Tg(olig2:EGFP)*^{vu12} (Shin et al., 2003) and *Tg(hsp70l:pard3-EGFP)*^{co14}.

Immunohistochemistry

Embryos and larvae were fixed in 4% paraformaldehyde (PFA) overnight at 4°C. Fixed embryos were imbedded in 1.5% agar with 5% sucrose and transferred to a 30% sucrose solution in scintillation vials and incubated at 4°C overnight. The blocks were then frozen and cut into 10-15 μm sections using a cryostat microtome. The sections were incubated with the following primary antibodies: rabbit anti-Sox10 (1:1,000) (Park et al., 2005), rabbit anti-phosphohistone H3 (1:1,000, #06-570, Millipore), mouse anti-BrdU (1:100, #G3G4, Developmental Studies Hybridoma Bank [DSHB]), rabbit anti-PkC (1:200, #sc-216, Santa Cruz Biotechnology, Inc.), mouse anti-Islet (1:1,000, clone # 39.4D5, DSHB,) mouse anti–ZO-1 (1:200, #33-9100, Invitrogen), rabbit anti-Sox2 (1:500, # ab997959, Abcam), rabbit anti-GFAP (1:100, #RB-087-A1, NeoMarkers), mouse anti– ZRF-1 (1:500, University of Oregon Monoclonal Antibody Facility). For fluorescent detection of antibody labeling we used Alexafluor 568 and Alexafluor 647 goat anti-mouse and goat anti-rabbit secondary antibodies (1:200, Invitrogen). F-actin was labeled using Rhodamine Phalloidin (1:100, Invitrogen) R-415). To detect EdU incorporation,

we incubated the slides in 250 μL of the EdU Detection Reaction mix (Invitrogen) for 40 minutes at room temperature. Images were collected on a Zeiss Axio Observer microscope equipped with a PerkinElmer UltraVIEW VoX spinning disk confocal system and Volocity imaging software (PerkinElmer). Images were contrast enhanced using either Volocity or Photoshop (Adobe CS4).

In situ RNA Hybridization

Embryos and larvae were fixed in 4% PFA overnight at 4°C and stored in methanol at -20°C. After linearizing plasmids with the appropriate restriction enzymes, antisense cRNA was transcribed using Roche digoxigenin-labeling reagents and T3, T7, or SP6 RNA polymerases (New England Biolabs). To detect *miR-219* expression, we used a *dre-miR-219* miRCURY LNA probes consisting of the sequence 5' AAGAATTGCGTTTGGACAATCA 3' (Exiqon 35172-01) (Kloosterman et al., 2006). After processing embryos for in situ RNA hybridization embryos were embedded in agar and sectioned as described above. Sections were rehydrated in 1X PBS for 30 min then covered with 75% glycerol. Images were obtained on a Zeiss Axio Observer microscope equipped with DIC optics, a Retiga Exi color camera and Volocity imaging software. Some images were contrast enhanced using Photoshop (Adobe CS4).

Luciferase Assay

A 220 bp sequence containing the predicted target site was placed in the 3' UTR of a renilla luciferase reporter gene using the commercially available plasmid, psiCHECK-2 (Promega). This construct and microRNA mimics were co-transfected into HEK293T cells using Lipofectamine 2000 (Invitrogen) following the vendor's protocol. The mimics used were miRIDIAN mimic

has-miR-219-5p (5'-UGAUUGUCCAAACGCAAUUCU-3') (Thermo Scientific CN-300575-05-0005), and miRIDIAN microRNA mimic negative control #1 list sequence (Thermo Scientific CN-001000-01-05). Both mimics were stored at -20°C at a 20 μM stock concentration and transfected at 0.08 μM. Luciferase activity was detected using the SpectraMax L Luminescence microplate reader (Molecular Devices).

Morpholino Injections

Antisense morpholino oligonucleotides were purchased from Gene Tools, LLC. These included: *miR-219* MO (5'-CAAGAATTGCGTTTGGACAATCA-3') (Zhao et al., 2010). *pard3* MO1 (5'-TCAAAGGCTCCCGTGCTCTGGTGTC-3') (Alexandre et al., 2010); (Tep et al., 2011), *pard3* Target Protector MO (5'-CTGATTGTCAGAGCATCTCTACTAC-3'), control *pard3* Target Protector (TP) MO (5'-ACAGAGTCAAAGTGACGGACTCC-3') and *prkci* TP MO (AAGCGACCGTCACACACTCCTCCGC). Morpholino oligonucleotides were dissolved in water to create stock solutions of 1 mM and diluted in 2X injection buffer (5 mg ml–1 Phenol red, 40 mM HEPES and 240 mM KCl) to create a working injection concentration of 0.25 mM. All morpholinos were co-injected with 0.09 mM dose of *p53* MO. We injected 1-2 nl into the yolk just below the single cell of fertilized embryos. All morpholino oligonucleotide injected embryos were raised in embryo medium at 28.5°C.

BrdU and EdU Labeling

Dechorionated embryos were labeled with 5-bromo-2′-deoxyuadine (BrdU) (Roche) by incubating them in 20 mM BrdU in Embryo Medium (EM) with 10% DMSO at room temperature for 30 min. For labeling with 5-ethynyl-2′-deoxyuridine (EdU), embryos were

incubated in 2 mM EdU (Click-iT EdU Alexafluor 555 detection kit, Invitrogen #c10338) in EM with 10% DMSO for 30 minutes at room temperature. The fish were then fixed in 4% PFA in PBS with 116 mM sucrose and 150 μM $CaCl_2$ at 4°C overnight.

GFP Injections and Quantification

1200 bp *pard3* and 800bp *prkci* UTRs were cloned in a EGFP containing vector, which was used as a template to transcribe mRNA with the EGFP located 5' of the UTRs. These mRNAs were injected with or without *miR-219* at one cell stage and raised to 30 hpf. Images of 20 embryos per group were collected using a Leica M165 FC microscope equipped with a SPOT RT3 camera and SPOT imaging software (Diagnostic Instruments Inc.). Embryos were placed in similar positions and images were collected under identical acquisition settings. After collection images were imported into ImageJ software and background readings were sampled from at least 10 images. The threshold was set to be double the background value and pixel intensity was collected in a set region of interest (ROI) of 170 x 33 pixels above the yolk extension. The experiment was performed three times and readings from 60 total embryos per group are reported.

***Tg(hsp70l:pard3-EGFP)* construction and Heat Shock Procedure**

The (*hsp70l:pard3-EGFP)* plasmid was constructed by first inserting *pard3-EGFP* cDNA (Geldmacher-Voss et al. 2003) into the middle entry vector of the Gateway Recombination Kit (Invitrogen). The resulting vector was recombined with *p5e-HSP70I* (Tol2 Kit plasmid #222), *p3E-PolyA* (Tol2 Kit plasmid #302), and *pDestTol2pA2* (Tol2 kit plasmid #394) through a Gateway LR recombination reaction. The (*hsp70I:pard3-GFP)* plasmid was co-injected with

Tol2 mRNA at 25ng/ul DNA and 25ng /ul RNA into newly fertilized eggs. Injected fish were raised to adulthood and screened for germline transmission. Founder fish were outcrossed to AB fish and F1 embryos were raised to adulthood.

53 hpf *Tg(hsp70l:pard3-EGFP)* and non-transgenic control embryos were placed in a 15mL conical tube in approximately 10mL of embryo medium and immersed in a 38°C water bath for one hour after which they were allowed to recover at RT for one hour. Heat shock was repeated 2-3 times before the embryos were placed in a Petri dish and allowed to develop at 32°C overnight. Embryos were fixed at 72 hpf and processed for immunohistochemistry as described above.

Quantitative PCR

RNA was isolated from 15-20 pooled larvae for each control or experimental condition. Samples for each condition were collected in triplicate. Reverse transcription was performed using the TaqMan MicroRNA Reverse Transcription Kit (PN 4366596, Applied Biosystems). Real time qPCR was performed in triplicate for each cDNA sample using an Applied Biosystems StepOne Plus machine and software version 2.1. TaqMan microRNA Assays were used to detect *miR-219* (assay ID 000522) and endogenous control *U6* snRNA (assay ID 001973).

Quantification and Statistical Analysis

Cell counts were obtained by direct observation of sections using the microscopes described above. For Sox10, Sox2, PH-3 and Isl quantification, 10 sections per embryo from 15 embryos per group with two or three replicates were counted to produce the average number per section. *P* values were generated using an unpaired *t*-test using GraphPad Prism software. Dorsally

migrated OPCs were assessed based on lateral views of *Tg(olig2:EGFP)* embryos at 3 dpf. *olig2:*EGFP positive cells were counted over the entire spinal cord. Larvae classified as normal had the number of dorsally migrated OPCs typical of wild type. Larvae were classified as severe if fewer than 5 OPCs had migrated and mild in all other circumstances. *P* values were generated using an unpaired *t*-test comparing the number of normal embryos when injected with *miR-219 MO* alone or *miR-219* together with *pard3* MO.

Supplemental References

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